

An indirect Spectrophotometric Methods for the estimation of Lamivudine in pure and tablet dosage form

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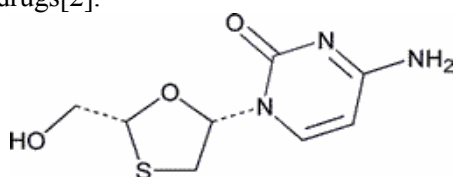
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Abstract : Lamivudine is an antiviral drug. Two simple, rapid and sensitive colorimetric methods have been developed for Lamivudine in pharmaceutical formulations. Method A is based on the formation of oxidative-coupling reaction involving the use of iron (III)-MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride). Method B is based on the formation of Schiff's base by using the p-Dimethyl Amino Benzaldehyde (Ehrlich reagent). These colored complexes show maximum absorption at 630nm for method A and 570nm for method B. The developed methods obey Beer's law in the concentration range of 0.5-20 µg/ml for method A and 5-30 µg/ml for method B respectively. The developed methods have been statistically validated for application in pharmaceutical quality control laboratory.

Key words: Lamivudine, 3-methyl-2-benzothiazolinone hydrazone hydrochloride, p-Dimethyl Amino Benzaldehyde, colorimeter.

Introduction:

Lamivudine is an antiviral agent. Lamivudine (3TC) is a cytosine analogue with potent activity against Human Immunodeficiency Virus (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity[1]. Lamivudine is used in treatment of HBV infections and it has been strongly recommended for the treatment of HIV infections in combination with other antiviral drugs[2].



Lamivudine

Lamivudine (LMV), [1-(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] cytosine] is highly active against Hepatitis B virus and HIV[3]. Literature survey revealed the availability of only few analytical methods such as UV spectrophotometry [4], visible spectrometry[5], HPLC for estimation of Lamivudine in pharmaceutical dosage forms[6,7].

The aim of present work is to find out a simple, specific, spectrophotometric method developed for the detection of lamivudine in bulk drug and pharmaceutical formulation.

Experimental

Instruments: Optical density measurements were made on Systronics Colorimeter 115.

Preparation of reagent solutions: All the reagents used were of analytical reagent grade.

1. MBTH reagent: About 200mg of MBTH was accurately weighed and dissolved in 20ml of distilled water. The final volume was made upto 100ml with distilled water.
2. FeCl₃ solution: 700mg of ferric chloride was dissolved in 100ml of 0.5% HCL.
3. PDAB reagent: 1gm of p-Dimethyl Amino Benzaldehyde was dissolved in 5ml of H₂SO₄ and 5 ml of distilled water.

Preparation of standard and sample solutions:

About 100mg of Lamivudine pure drug or a weight of formulation equivalent to 100mg of Lamivudine was accurately weighed, transferred into 100ml of volumetric flask and dissolved in 100ml of methanol. The solution was further diluted to 100μg/ml of Lamivudine.

Assay procedure:

Method A: Volumes of standard lamivudine solution (1ml=10μg) ranging from 0.5ml to 6ml

were transferred in to series of 10 ml volumetric flasks. To each volumetric flask added 1.5ml of MBTH reagent and 1.0ml of FeCl₃ solution and mixed well. The final volume was made upto 10ml with methanol and allow to stand for 10min. The blue coloured complex was observed and absorbances were measured at 630 nm against the reagent blank. The amount of lamivudine present in the sample solution was computed from its calibration curve.

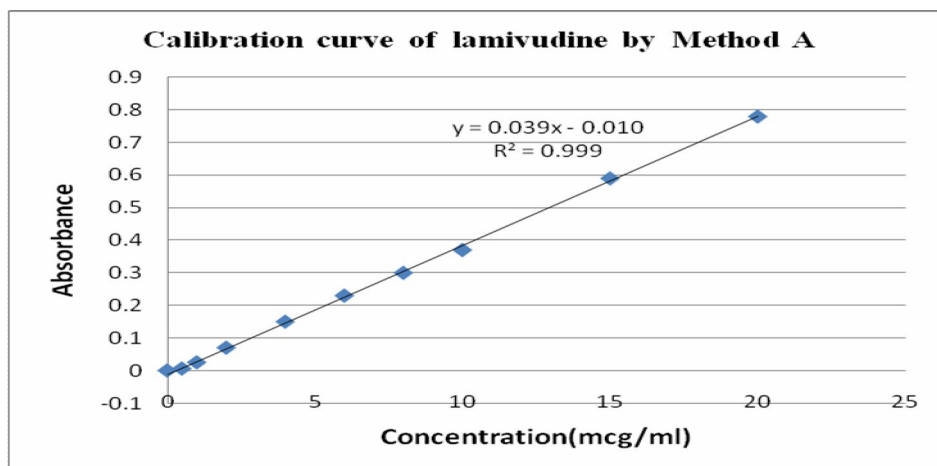
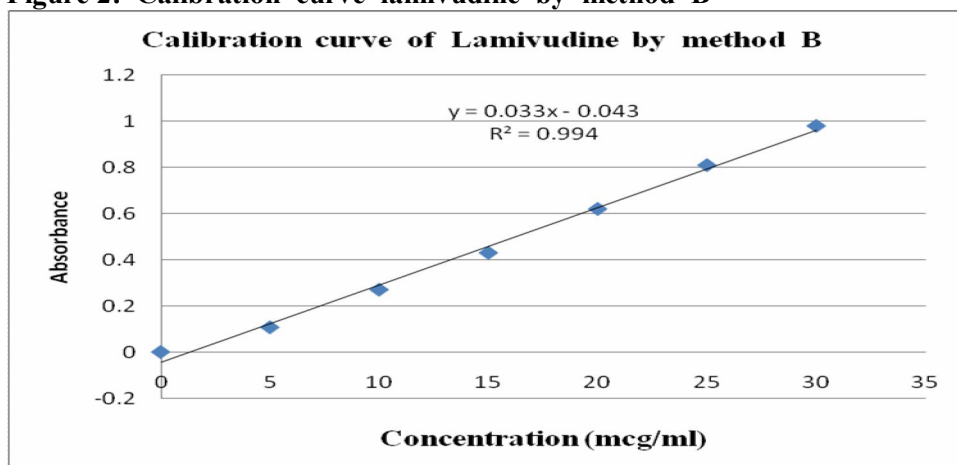
Method B: Aliquots of standard lamivudine solution (1ml=100μg) ranging from 0.5ml to 3ml were transferred into a series of 10ml volumetric flasks. To each volumetric flask added 2ml of PDAB reagent and 0.3ml of toluene and 0.02ml of conc. H₂SO₄ and mixed well. The final volume was made upto 10ml with methanol. The rose red coloured complex was observed and absorbances were measured at 570nm against the reagent blank. The amount of lamivudine present in the sample solution was computed from its calibration curve.

Table-1: Optical characteristics and other parameters of Lamivudine

Parameter	Method A	Method B
λ _{max} (nm)	630	570
Beer's law(μg/ml)	0.5-20	5-30
Sandell's sensitivity	0.026	0.0467
Molar absorptivity (Litre/mole ⁻¹ /cm ⁻¹)	301×10 ²	619×10 ²
%RSD	0.652	0.541
Correlation coefficient(r)	0.999	0.998
Regression equation(y)		
Slope(a):	0.039	0.035
Intercept(b):	0.012	0.081

Table-2: Estimation of Lamivudine in pharmaceutical dosage forms

Formulation	Labelled amount(mg)	Amount found(mg)		%Recovery	
		Method A	Method B	Method A	Method B
Tablet-1	150	150.03	149.62	100.02	99.75
Tablet-2	300	300.12	299.73	100.04	99.91

Figure 1: Calibration curve of lamivudine by Method A**Figure 2: Calibration curve lamivudine by method B**

Results and Discussion:

Lamivudine is available as 150 and 300mg of tablets. Lamivudine contains primary amino group in its structure. By exploiting the some chemical properties of the primary amino functional group, two simple colorimetric methods have been developed (Table-1).

The formation of oxidative-coupling reaction between primary amine and MBTH involving the use of iron (III). The developed method A is based on oxidative coupling of drug with MBTH. The calibration curve of lamivudine by MBTH was depicted in Figure-1.

The reaction of primary amine with carbonyl compounds forms the condensation products are referred to as Schiff's bases. The primary amine group which is present in the

structure of the drug reacts with the PDAB reagent and forms rose red colour. The calibration curve of lamivudine by PDAB was depicted in Figure-2.

The results obtained for the determination of lamivudine in pharmaceutical formulations (tablets) by the proposed methods (Table-2). The obtained results are in good agreement with each other. Interference studies revealed that the common excipients usually present in the dosage form do not interfere in the proposed procedure.

The results indicate that the proposed methods are simple, reproducible and accurate and can be used for the routine determination of lamivudine in bulk and pharmaceutical dosage forms.

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